MONOSCREEN Quant ELISA

Bovine gamma interferon

ELISA kit for the semi-quantitative determination
of Bovine gamma interferon
Sandwich test for blood sera and plasma
Diagnostic test for cattle
Monowell

I - PRINCIPLE OF THE TEST

The test uses 96-well microtitration plates sensitised by a specific monoclonal antibody for the bovine gamma Interferon. This antibody allows a specific capture of the bovine gamma Interferon which is present in the samples (plasma or serum). The entire plate has been sensitized with this antibody.
Samples are applied without dilution and incubated on the microplate for 1 hour at 21°C +/- 3°C. A reference curve must be prepared by dilution of the standard purchased with the kit. The standard is a supernatant of bovine lymphocytes stimulated "in vitro" by concanavalin A. As no international standard exists for gamma interferon, the standard of the kit is calibrated in arbitrary units.
After this first incubation step, the plate is washed and incubated for 1 hour with the conjugate - a peroxidase labelled anti-bovine gamma Interferon specific antibody. After this second incubation, the plate is washed again and the chromogen (tetramethylbenzidine TMB) is added. This chromogen has the advantage of being more sensitive than the other peroxidase chromogens and of not being carcinogenic.
Enzymatic reaction can be stopped by acidification and the resulting optical density at 450 nm can be recorded using a photometer.
The optical density obtained for unknown samples is reported on the calibration curve in order to calculate the IFN concentration.

II - COMPOSITION OF THE KIT

- **Microplates**: Two 96-well microtitration plates. The whole plate is sensitised by anti-Bovine gamma Interferon specific antibodies.
- **Washing solution**: One 100- ml bottle of 20x concentrated washing solution. The solution crystallises spontaneously at low temperatures. If only part of the solution is to be used, bring the bottle to 21°C +/- 3°C until disappearance of all crystals. Mix the solution well and remove the necessary volume. Dilute the buffer 1:20 with distilled or demineralised water. Store the diluted solution between +2°C and +8°C.
- **Dilution buffer**: One 50-ml bottle of 5x colored, concentrated buffer for diluting of standard and conjugate. Dilute this concentrated dilution buffer 1:5 with distilled or demineralised water. Store the diluted solution between +2°C and +8°C. If a deposit forms at the bottom of the container filter the solution on Whatman filter paper.

- **Conjugate**: One 0,5 ml bottle of anti-bovine gamma Interferon-peroxidase conjugate. The conjugate will keep between +2°C and +8°C. This reagent must be diluted 50-fold in the dilution buffer. –
- **Bovine gamma Interferon standard**: 2 bottles containing the Bovine gIFN standard. Reconstitute this standard with 0.5 ml of distilled or demineralised water. The reconstituted standard may be kept at -20°C. Divide the reconstituted standard into several portions before freezing in order to avoid repeated freezing and thawing. If these precautions are taken the reagent may be kept for several months.
- **Single component TMB**: One 25-ml bottle of the chromogen tetramethylbenzidine (TMB). Store between +2°C. and +8°C. protected from the light. The reagent is ready to use.
- **Stopping solution**: One 15-ml bottle of the 1 M phosphoric acid stop solution.

	BIO K 093/2
Microplaques	2
Washing solution	1 x 100 ml (20x)
Colored Dilution buffer	1 x 50 ml (5x)
Conjugate	1 x 0,5 ml (50x)
Standard	2 x 0,5 ml (lyophilisé)
Single component TMB	1 x 25 ml (1x)
Stop solution	1 x 15 ml (1x)

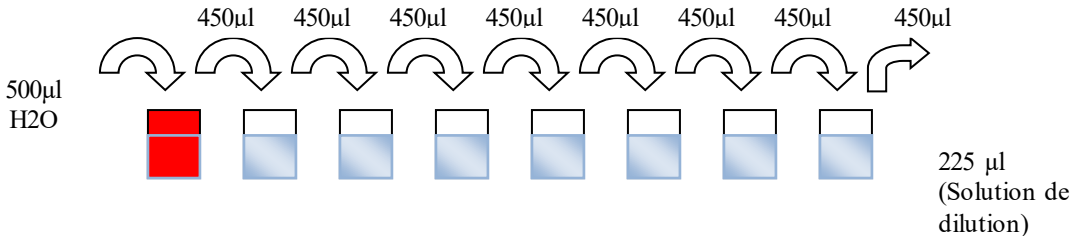
III - PRECAUTIONS FOR USE

- This test may be used for "in vitro" diagnosis only. It is strictly for veterinary use.
- The reagents must be kept between +2°C and +8°C. The reagents cannot be guaranteed if the shelf-life dates have expired or if they have not been kept under the conditions described in this insert.
- The concentrated wash solution and dilution buffer may be stored at room temperature. Once diluted, these solutions remain stable for six weeks if kept between +2°C and +8°C.
- Unused strips must be stored immediately in the aluminium envelope, taking care to keep the desiccant dry and the envelope's seal airtight. If these precautions are taken, the strips' activity can be conserved up to the kit's shelf-life date.
- Do not use reagents from other kits.
- The quality of the water used to prepare the various solutions is of the utmost importance. Do not use water that may contain oxidants (e.g., sodium hypochlorite) or heavy metal salts, as these substances can react with the chromogen.
- Discard all solutions contaminated with bacteria or fungi.
- The stop solution contains 1 M phosphoric acid. Handle it carefully.
- All materials and disposable equipment that come in contact with the samples must be considered potentially infectious and be disposed of in compliance with the legislation in force in the country.
- To guarantee the reliability of the results, one must follow the protocol to the letter. Special care must be taken in observing the incubation times and temperatures, as well as measuring the volumes and dilutions accurately.

IV – PROCEDURE

1- Bring all the reagents to 21°C +/- 3°C before use.
2- Dilute the concentrated washing solution 20 fold in distilled water. Be sure that all crystals have disappeared before dilution.
   Dilute the concentrated Dilution buffer 5 fold in distilled water.
   Keep these solutions between +2°C and +8°C when not used.
3- Prepare the calibration curve as follows: resuspend the contents of one bottle of standard in 500 µl of distilled water. Make 7 dilutions (of the order 1,5) of the standard in the dilution solution. The precision of the ELISA test depends greatly on the careful preparation of the dilutions. In order to obtain reliable results, it is highly recommended to use precision dilution equipment such Hamilton syringes.
   If a manual pipette is used, the tip must be changed between dilutions.

	Dilution	Concentration
Stock solution	1/1	100 UA/ml
1° dilution	1/1,5	66,66 UA/ml
2° dilution	1/2,25	44,44 UA/ml
3° dilution	1/3,375	29,63 UA/ml
4° dilution	1/5,0625	19,75 UA/ml
5° dilution	1/7,59375	13,17 UA/ml
6° dilution	1/11,390625	8,78 UA/ml
7° dilution	1/17,0859375	5,85 UA/ml



Distribute the 8 dilutions in duplicate on the microplate (100 µl per well).

4- Distribute the unknown samples and undiluted on the microplate (100 µl per well).
5- Cover with a lid and incubate the plate at 21°± 3°C for one hour.
6- Rinse the plate with the washing solution prepared as instructed in the section "Composition of the Kit". To do this, dispose of the microplate's contents by flipping it sharply over a container filled with an inactivating agent. Let the microplate drain upside-down on a sheet of clean absorbent paper so as to eliminate all liquid. Add 300 µl of the washing solution, and then empty the plate once again by flipping it over above the containment vessel. Repeat the entire operation twice, taking care to avoid the formation of bubbles in the microwells. After the plate has been washed three times proceed to the next step.
7- Dilute the conjugate 1:50 with the dilution buffer (for example, for one plate dilute 250 µl of the conjugate stock solution in 12.25 ml of diluent). Add 100 µl of the diluted conjugate solution to each well. Cover with a lid and incubate the plate at 21°± 3°C for one hour.
8- Wash the plate as described in §6 above.
9- Add 100 µl of the TMB solution to each well on the plate. The TMB solution must be absolutely colourless when it is pipetted into the wells. If a blue colour is visible, this means that the solution in the pipette has been contaminated.
10 -Incubate for 10 minutes at 21°C +/- 3°C. This time is given as a guideline only, for in some circumstances it may be useful to lengthen or shorten the incubation time.
11 -Add 50 µl of stop solution per microwell. The blue colour will change into a yellow colour.
12 -Read the optical densities in the microwells using a plate reader and a 450 nm filter. Results must be read fairly soon after the stopping solution has been added since the chromogen may cristallize in wells with strong signals and thereby distort the data.

V – INTERPRETING THE RESULTS

In order to calculate the concentrations of gIFN in the unknown samples, it is preferable to use a computer program with curve fitting options of the type Log/Logit or 4 parameter. The Deltasoft program of Biometallics incorporated (P.O. Box 2251 Princeton, NJ USA Tel: 08543 609.275.0133 Fax 609.275.9485) is particularly well adapted to this kit.
If such a program is available, introduce the 8 gIFN concentrations in the standard curve (2 values per dilution). Name each sample and indicate its dilution factor. The program will determine the 4 parameters of the standard curve with its correlation coefficient.
Interpolate the values in order to obtain the concentrations of the unknown samples.

If a program such as Deltasoft is not available, one can determine the concentrations of the unknown samples using a graphic method, as described below.

Use the graph provided with the kit. The bold vertical lines correspond to the concentrations of the standard curve (100 UA/ml – 5,85 UA/ml). Calculate for each of the 8 points of the calibration curve the mean of the optical densities. Place on each of the 8 vertical lines the values obtained. Draw the curve so that it best fits the 8 experimental points.
For each of the unknown samples, place the optical densities for each sample on the ordinate and draw the corresponding horizontal lines. At the point of intersection of the horizontal lines with the curve, draw the vertical lines and determine the concentration of the samples on the abscissa (UA/ml).

X – ORDERING INFORMATION

QuantELISA Bovine gamma interferon 2 X 80 tests BIO K 093/2